

AMENDMENTS TO THE CLAIMS

1-143. (canceled)

144. (New) An isolated polynucleotide molecule comprising an operably linked i) transcriptional promoter operative in a mammalian cell or operative in vitro, ii) a polynucleotide sequence encoding a human PIV genome and comprising at least one sequence selected from the group consisting of the complement of nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73,

or encoding a human PIV antigenome and comprising at least one nucleotide sequence selected from the group consisting of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73, and

iii) a transcriptional terminator operative in a mammalian cell or operative in vitro.

145. (New) The isolated polynucleotide molecule of claim 144, in which the polynucleotide ii) encodes a human PIV3 genome or antigenome.

146. (New) An isolated polynucleotide molecule comprising an operably linked i) transcriptional promoter operative in a mammalian cell or operative in vitro, ii) a polynucleotide sequence encoding a human PIV genome and comprising at least one sequence selected from the complement of nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 69, 71 and 73,

or encoding a human PIV antigenome and comprising at least one sequence selected from the group consisting of nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 69, 71 and 73, and

iii) a transcriptional terminator operative in a mammalian cell or operative in vitro.

147. (New) The isolated polynucleotide of claim 146, in which the polynucleotide ii) is a polynucleotide sequence encoding a human PIV3 genome and comprising the complement of nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 69, 71 and 73,

or encoding a human PIV3 antigenome and comprising the nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 69, 71 and 73.

148. (New) An isolated polynucleotide molecule comprising an operably linked transcriptional promoter operative in a mammalian cell or operative in vitro, a polynucleotide sequence encoding a chimeric partial or complete PIV genome or antigenome that confers a phenotype of attenuation of replication of at least 10-fold in the respiratory tract of a subject infected with a virus comprising said chimeric partial or complete partial or complete PIV genome or antigenome, and a transcriptional terminator operative in a mammalian cell or operative in vitro,

wherein said polynucleotide sequence encoding said chimeric partial or complete PIV genome or antigenome comprises a polynucleotide sequence of a background partial or complete PIV genome or antigenome and at least one heterologous PIV sequence selected from a HPIV 1 sequence, a HPIV 2 sequence, a HPIV 3 sequence, a BPIV sequence or a MPIV sequence,

and wherein said polynucleotide sequence encoding said chimeric partial or complete PIV genome or antigenome comprises a polynucleotide encoding the L protein of the wild-type of said background PIV or of said heterologous PIV.

149. (New) The isolated polynucleotide of claim 148, in which the polynucleotide sequence encoding the background partial or complete PIV genome or antigenome further includes at least one mutation at a position corresponding to a position in the genome of HPIV3 selected from the group consisting of Val96 of the N protein, Ser389 of the N protein, Ile96 of the C protein, Ile420 of the F protein, Ala450 of the F protein, Val384 of the HN protein, nucleotide 23 of the 3' leader sequence, nucleotide 24 of the 3' leader sequence, nucleotide 28 of the 3' leader sequence, nucleotide 45 of the 3' leader sequence and nucleotide 62 in the N gene start sequence.

150. (New) The isolated polynucleotide of claim 148, in which the chimeric partial or complete PIV genome or antigenome comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein or of a F glycoprotein.

151. (New) The isolated polynucleotide of claim 149, in which the chimeric partial or complete PIV genome or antigenome comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein or of a F glycoprotein.

152. (New) The isolated polynucleotide of claim 149, in which the heterologous PIV sequence is from HPIV1.

153. (New) The isolated polynucleotide of claim 150, in which the heterologous PIV sequence is from HPIV1.

154. (New) The isolated polynucleotide of claim 148, that comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein and a heterologous PIV sequence that encodes an open reading frame of a F glycoprotein.

155. (New) The isolated polynucleotide of claim 149, that comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein and a heterologous PIV sequence that encodes an open reading frame of a F glycoprotein.

156. (New) The isolated polynucleotide of claim 150, that comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein and a heterologous PIV sequence that encodes an open reading frame of a F glycoprotein.

157. (New) The isolated polynucleotide of claim 154, in which both of the heterologous PIV sequences are from HPIV1.

158. (New) The isolated polynucleotide of claim 155, in which both of the heterologous PIV sequences are from HPIV1.

159. (New) The isolated polynucleotide of claim 156, in which the heterologous PIV sequence is a gene segment encoding a portion of an open reading frame of a HN or F glycoprotein comprising an antigenic domain or epitope or the cytoplasmic tail portion of said HN or F glycoprotein and replaces the counterpart gene segment of said background PIV sequence to form an open reading frame encoding a chimeric glycoprotein.

160. (New) An isolated polynucleotide molecule comprising an operably linked transcriptional promoter operative in a mammalian cell or operative in vitro, a polynucleotide sequence encoding a chimeric partial or complete PIV genome or antigenome that includes at least one point mutation that confers a phenotype of attenuation of replication of at least 10-fold in the respiratory tract of a subject infected with a virus comprising said partial or complete PIV genome or antigenome, and a transcriptional terminator operative in a mammalian cell or operative in vitro,

wherein said polynucleotide sequence encoding said partial or complete PIV genome or antigenome comprises a polynucleotide sequence of a background partial or complete PIV genome or antigenome and at least one heterologous PIV sequence selected from a HPIV 1 sequence, a HPIV 2 sequence, a HPIV 3 sequence a BPIV sequence or a MPIV sequence,

and wherein said polynucleotide sequence encoding said partial or complete PIV genome or antigenome comprises a polynucleotide encoding the L protein of the wild-type of said background PIV or of said heterologous PIV.

161. (New) The isolated polynucleotide of claim 160, in which the chimeric partial or complete PIV genome or antigenome comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein or of a F glycoprotein.

162. (New) The isolated polynucleotide of claim 160, in which the heterologous PIV sequence is from HPIV1.

163. (New) The isolated polynucleotide of claim 161, in which the heterologous PIV sequence is from HPIV1.

164. (New) The isolated polynucleotide of claim 160, that comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein and a heterologous PIV sequence that encodes a F glycoprotein.

165. (New) The isolated polynucleotide of claim 164, in which both of the heterologous PIV sequences are from HPIV1.

166. (New) An isolated polynucleotide molecule comprising an operably linked transcriptional promoter operative in a mammalian cell or operative in vitro, a polynucleotide sequence encoding a chimeric partial or complete PIV genome or antigenome, and a transcriptional terminator operative in a mammalian cell or operative in vitro, wherein said polynucleotide sequence encoding said chimeric partial or complete PIV genome or antigenome comprises a polynucleotide sequence encoding a background partial or complete PIV genome or antigenome and at least one heterologous PIV polynucleotide sequence selected from a HPIV 1 sequence, a HPIV 2 sequence, a HPIV 3 sequence, a BPIV sequence or a MPIV sequence,

wherein said heterologous PIV polynucleotide sequence comprises a transcription unit comprising a polynucleotide sequence encoding an open reading frame of a protein of said heterologous PIV or portion thereof providing an antigenic determinant that is inserted between a gene start sequence and a gene end sequence of the PIV of the background.

167. (New) The isolated polynucleotide of claim 166, in which the heterologous PIV polynucleotide sequence replaces an open reading frame of a gene of the background PIV.

168. (New) The isolated polynucleotide of claim 166, in which the heterologous PIV nucleotide sequence is added to the genome or antigenome of the background PIV.

169. (New) The isolated polynucleotide of claim 166, in which the polynucleotide sequence encoding the background partial or complete PIV genome or antigenome further includes at least one mutation at a position corresponding to a position in the genome of HPIV3 selected from the group consisting of Val96 of the N protein, Ser389 of the N protein, Ile96 of the C protein, Ile420 of the F protein, Ala450 of the F protein, Val384 of the HN protein, Tyr942 of the L protein, Leu992 of the L protein, Thr1558 of the L protein, nucleotide 23 of the 3' leader sequence, nucleotide 24 of the 3' leader sequence, nucleotide 28 of the 3' leader sequence, nucleotide 45 of the 3' leader sequence and nucleotide 62 in the N gene start sequence.

170. (New) The isolated polynucleotide of claim 166, in which the polynucleotide sequence encoding the background partial or complete PIV genome or antigenome further includes mutations at positions corresponding to a position in the genome of HPIV3 of Tyr942 of the L protein, Leu992 of the L protein and Thr1558 of the L protein.

171. (New) The isolated polynucleotide of claim 166, in which the polynucleotide sequence that encodes the background partial or complete PIV genome comprises at least one nucleotide sequence that is the complement of a sequence selected from the group consisting of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73

or in which the polynucleotide sequence that encodes the background PIV antigenome comprises at least one nucleotide sequence selected from the group consisting of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73.

172. (New) The isolated polynucleotide of claim 167, in which the polynucleotide sequence that encodes the background partial or complete PIV genome comprises the complement of nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73,

or in which the polynucleotide sequence that encodes the background PIV antigenome comprises nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73.

173. (New) The isolated polynucleotide of claim 168, in which the polynucleotide sequence that encodes the background partial or complete PIV genome comprises the complement of nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73,

or in which the polynucleotide sequence that encodes the background PIV antigenome comprises nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73.

174. (New) The isolated polynucleotide of claim 167, in which the chimeric partial or complete PIV genome or antigenome comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein or of a F glycoprotein.

175. (New) The isolated polynucleotide of claim 174, in which the heterologous PIV sequence is from HPIV1.

176. (New) The isolated polynucleotide of claim 166, that comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein and a heterologous PIV sequence that encodes an open reading frame of a F glycoprotein.

177. (New) The isolated polynucleotide of claim 176, in which both of the heterologous PIV sequences are from HPIV1.

178. (New) The isolated polynucleotide of claim 166, in which the heterologous PIV sequence is a gene segment encoding a portion of an open reading frame of a HN or F glycoprotein comprising an antigenic domain or epitope or the cytoplasmic tail portion, of said

HN or F glycoprotein and replaces the counterpart gene segment of said background PIV sequence to form an open reading frame encoding a chimeric glycoprotein.

179. (New) An infectious chimeric parainfluenza virus (PIV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a human partial or complete PIV genome comprising at least one sequence selected from the group consisting of the complement of nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73,

or a human PIV antigenome comprising at least one nucleotide sequence selected from the group consisting of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73.

180. (New) The infectious chimeric PIV of claim 179, in which the human partial or complete PIV genome or antigenome is a human PIV3 genome or antigenome.

181. (New) An infectious chimeric parainfluenza virus (PIV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a human partial or complete PIV genome comprising at least one sequence selected from the complement of nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 69, 71 and 73, or a human PIV antigenome comprising at least one sequence selected from the group consisting of nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 69, 71 and 73.

182. (New) The infectious chimeric PIV of claim 181, in which the human partial or complete PIV genome or antigenome is a human PIV3 genome comprising the complement of nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 69, 71 and 73, or a human PIV3 antigenome comprising the nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 69, 71 and 73.

183. (New) An infectious chimeric parainfluenza virus (PIV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and

a chimeric partial or complete PIV genome or antigenome comprising a a background partial or complete PIV genome or antigenome and at least one heterologous PIV sequence selected from a HPIV 1 sequence, a HPIV 2 sequence, a HPIV 3 sequence a BPIV sequence or a MPIV sequence,

wherein said said chimeric partial or complete PIV genome or antigenome comprises a polynucleotide encoding the L protein of the wild-type of said background PIV or of said heterologous PIV,

said infectious chimeric PIV being attenuated for replication at least 10-fold in the respiratory tract of a subject infected with a virus.

184. (New) The infectious chimeric PIV of claim 183, in which the background partial or complete PIV genome or antigenome further includes at least one mutation at a position corresponding to a position in the genome of HPIV3 selected from the group consisting of Val96 of the N protein, Ser389 of the N protein, Ile96 of the C protein, Ile420 of the F protein, Ala450 of the F protein, Val384 of the HN protein, nucleotide 23 of the 3' leader sequence, nucleotide 24 of the 3' leader sequence, nucleotide 28 of the 3' leader sequence, nucleotide 45 of the 3' leader sequence and nucleotide 62 in the N gene start sequence.

185. (New) The infectious chimeric PIV of claim 183, in which the chimeric partial or complete PIV genome or antigenome comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein or of a F glycoprotein.

186. (New) The infectious chimeric PIV of claim 184, in which the chimeric partial or complete PIV genome or antigenome comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein or of a F glycoprotein.

187. (New) The infectious chimeric PIV of claim 184, in which the heterologous PIV sequence is from HPIV1.

188. (New) The infectious chimeric PIV of claim 185, in which the heterologous PIV sequence is from HPIV1.

189. (New) The infectious chimeric PIV of claim 183, in which the chimeric partial or complete PIV genome or antigenome comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein and a heterologous PIV sequence that encodes an open reading frame of a F glycoprotein.

190. (New) The infectious chimeric PIV of claim 184, in which the chimeric partial or complete PIV genome or antigenome comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein and a heterologous PIV sequence that encodes an open reading frame of a F glycoprotein.

191. (New) The infectious chimeric PIV of claim 185, in which the chimeric partial or complete PIV genome or antigenome comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein and a heterologous PIV sequence that encodes an open reading frame of a F glycoprotein.

192. (New) The infectious chimeric PIV of claim 189, in which both of the heterologous PIV sequences are from HPIV1.

193. (New) The infectious chimeric PIV of claim 190, in which both of the heterologous PIV sequences are from HPIV1.

194. (New) The infectious chimeric PIV of claim 191, in which the heterologous PIV sequence is a gene segment encoding a portion of an open reading frame of a HN or F glycoprotein comprising an antigenic domain or epitope or the cytoplasmic tail portion of said HN or F glycoprotein and replaces the counterpart gene segment of said background PIV sequence to form an open reading frame encoding a chimeric glycoprotein.

195. (New) An infectious chimeric PIV virus comprising a chimeric partial or complete PIV genome or antigenome that includes at least one point mutation that confers a phenotype of attenuation of replication of at least 10-fold in the respiratory tract of a subject infected with a virus comprising said chimeric partial or complete PIV genome or antigenome,

wherein said chimieric partial or complete PIV genome or antigenome comprises a polynucleotide sequence of a background partial or complete PIV genome or antigenome and at least one heterologous PIV sequence selected from a HPIV 1 sequence, a HPIV 2 sequence, a HPIV 3 sequence a BPIV sequence or a MPIV sequence,

and wherein said polynucleotide sequence encoding said partial or complete PIV genome or antigenome comprises a polynucleotide encoding the L protein of the wild-type of said background PIV or of said heterologous PIV.

196. (New) The infectious chimeric PIV of claim 195, in which the chimeric partial or complete PIV genome or antigenome comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein or of a F glycoprotein.

197. (New) The infectious chimeric PIV of claim 195, in which the heterologous PIV sequence is from HPIV1.

198. (New) The infectious chimeric PIV of claim 196, in which the heterologous PIV sequence is from HPIV1.

199. (New) The infectious chimeric PIV of claim 195, in which the chimeric partial or complete PIV genome or antigenome comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein and a heterologous PIV sequence that encodes a F glycoprotein.

200. (New) The infectious chimeric PIV of claim 199, in which both of the heterologous PIV sequences are from HPIV1.

201. (New) An infectious chimeric PIV molecule comprising a chimeric partial or complete PIV genome or antigenome,

wherein said chimeric partial or complete PIV genome or antigenome comprises a background partial or complete PIV genome or antigenome and at least one heterologous PIV polynucleotide sequence selected from a HPIV 1 sequence, a HPIV 2 sequence, a HPIV 3 sequence a BPIV sequence or a MPIV sequence,

wherein said heterologous PIV polynucleotide sequence comprises a transcription unit comprising a polynucleotide sequence encoding an open reading frame of a protein of said heterologous PIV or portion thereof providing an antigenic determinant that is inserted between a gene start sequence and a gene end sequence of the PIV of the background.

202. (New) The infectious chimeric PIV of claim 201, in which the heterologous PIV polynucleotide sequence replaces an open reading frame of a gene of the background PIV.

203. (New) The infectious chimeric PIV of claim 201, in which the heterologous PIV nucleotide sequence is added to the genome or antigenome of the background PIV.

204. (New) The infectious chimeric PIV of claim 201, in which the background partial or complete PIV genome or antigenome further includes at least one mutation at a position corresponding to a position in the genome of HPIV3 selected from the group consisting of Val96 of the N protein, Ser389 of the N protein, Ile96 of the C protein, Ile420 of the F protein, Ala450 of the F protein, Val384 of the HN protein, Tyr942 of the L protein, Leu992 of the L protein, Thr1558 of the L protein, nucleotide 23 of the 3' leader sequence, nucleotide 24 of the 3' leader sequence, nucleotide 28 of the 3' leader sequence, nucleotide 45 of the 3' leader sequence and nucleotide 62 in the N gene start sequence.

205. (New) The infectious chimeric PIV of claim 201, in which the background partial or complete PIV genome or antigenome further includes mutations at positions corresponding to a position in the genome of HPIV3 of Tyr942 of the L protein, Leu992 of the L protein and Thr1558 of the L protein.

206. (New) The infectious chimeric PIV of claim 201, in which the background partial or complete PIV genome comprises at least one nucleotide sequence that is the complement of a sequence selected from the group consisting of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73

or in which the background PIV antigenome comprises at least one nucleotide sequence selected from the group consisting of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73.

207. (New) The infectious chimeric PIV of claim 202, in which background partial or complete PIV genome comprises the complement of nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73,

or in which the background PIV antigenome comprises nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73.

208. (New) The infectious chimeric PIV of claim 203, in which the background partial or complete PIV genome comprises the complement of nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73,

or in which the background PIV antigenome comprises nucleotide sequences of SEQ ID NOS: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73:

209. (New) The infectious chimeric PIV of claim 202, in which the chimeric partial or complete PIV genome or antigenome comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein or of a F glycoprotein.

210. (New) The infectious chimeric PIV of claim 209, in which the heterologous PIV sequence is from HPIV1.

211. (New) The infectious chimeric PIV of claim 201, in which the chimeric partial or complete PIV genome or antigenome comprises a heterologous PIV sequence that encodes an open reading frame of a HN glycoprotein and a heterologous PIV sequence that encodes an open reading frame of a F glycoprotein.

212. (New) The infectious chimeric PIV of claim 211, in which both of the heterologous PIV sequences are from HPIV1.

213. (New) The infectious chimeric PIV of claim 201, in which the heterologous PIV sequence is a gene segment encoding a portion of an open reading frame of a HN or F glycoprotein comprising an antigenic domain or epitope or the cytoplasmic tail portion of said HN or F glycoprotein and replaces the counterpart gene segment of said background PIV sequence to form an open reading frame encoding a chimeric glycoprotein.

214. (New) An immunogenic composition comprising the infectious chimeric PIV of any one of claims 179-213.

215. (New) A method for making a chimeric PIV, comprising expressing the isolated polynucleotide of any one of claims 144-178 in a cell or in a cell-free lysate, said cell or cell-free lysate comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P) and a large polymerase protein (L) of a human or bovine PIV.